APPENDIX III

PROCEDURE FOR ANALYZING NICOTINE SAMPLES OUTSIDE THE RANGE OF 0.1 TO 1.5 MILLIGRAMS PER CIGARETTE

1. Procedure to measure nicotine in samples containing more than 1.5 mg/cig.

   1.1 No changes in the methodology are needed except that the sample should be diluted with extracting solution until the analytical result is less than 1.5 mg/cig. The result is then multiplied by the dilution factor to determine the nicotine level.

   Example:
   A sample is analyzed and the nicotine result is 2.6 mg/cig. From this it appears that the sample should be diluted one to two. Pipette a 5 mL aliquot of the sample into a 10 mL volumetric flask and dilute to volume with extracting solution. Mix well. Analyzing the diluted sample yielded a result of 0.88 mg/cig. Multiply this result by the dilution factor of two to obtain the final result of 1.76 mg/cig.

2. Procedure to determine nicotine in samples containing between 0.01 and 0.2 mg/cig. nicotine.

   2.1 The changes in the methodology needed to analyze samples in this range are: (1) the extracting solution, (2) the standards, and (3) the electrometer attenuator setting for the nitrogen/phosphorous detector.

   2.2 Extracting Solution
   Reduce quinoline to 0.5 mL per gallon of isopropanol. Reduce methanol to 4 mL per gallon. Stopper and shake well. Concentration = quinoline 146 µg/mL and methanol 850 µg/mL.

2.3 Nicotine standards:

2.3.1 Using the nicotine stock standards made in Section 7.3.2, pipette 5 mL of each standard into 50 mL volumetric flasks and dilute to volume with isopropanol that does not contain any internal standards. Concentration = 50, 250, 500 and 1000 µg/mL.

Label: Nicotine Stock Standard
Concentration -
Date -
Technician -

2.3.2 Pipette 2 mL of each Stock Standard into four extraction vials which contain two whole and two quarter Cambridge pads. Prepare a fifth vial as a blank. Add 20 mL of extracting solution to each vial. Stopper and shake 15 minutes on the up and down extractor. Concentration = 100, 500, 1000, and 2000 µg per vial which is equal to (assuming five cigarettes smoked per pad and two pads are extracted/vial) 0.01, 0.05, 0.10 and 0.20 mg/cig.

Label: Nicotine Working Standard
Concentration -
Date -
Technician Name -

2.3.4 Transfer an aliquot from each vial to an auto sampler vial and seal. Set the auto injector to "maximum" wash between samples.

2.3.5 Standardize the instrument as outlined in this methodology, Section 8. Adjust the attenuator as necessary.

2.3.6 Analyze samples as outlined in Section 9.8.

3. Procedure to analyze nicotine in samples containing between 0.001 and 0.02 mg/cig.

3.1 To analyze samples at this level, the instrument must be operated near its maximum sensitivity settings. The baseline from the nitrogen/phosphorous detector will be "noisy". All glassware must be thorougly rinsed with distilled water because detergent residues may contain phosphorous compounds which would interfere with the N/P detector.

3.2 The changes in methodology necessary to analyze samples in this range are: (1) extracting solution, (2) standards, (3) electrometer settings and (4) autosampler set-up.

3.3 Extracting Solution

Reduce quinoline to 0.05 mL per gallon of isopropanol. There is no need to add an internal standard for water because this method cannot measure water at very low levels. Use the Karl Fischer method to measure water at these levels.

3.4 Nicotine Standards

3.4.1 Using the nicotine stock standards made in Section 7.3.2, pipette 1 mL of each standard into 100 mL volumetric flasks and dilute to volume with isopropanol that does not contain any internal standard. Concentration = 5, 25, 50, and 100 µg/mL.

Label: Nicotine Stock Standard
Concentration -
Date -
Technician Name -

3.4.2 Pipette 2 mL of each Stock Standard into four extraction vials which contain two whole and two quarter Cambridge pads. Prepare a fifth vial also containing Cambridge pads as a blank. Add 20 mL of extracting solution to each vial. Stopper and shake 15 minutes on the "up and down" extractor. Concentration equals (assuming five cigarettes smoked per pad and two pads are extracted per vial) 0.001, 0.005, 0.01 and 0.02 mg/cig.

Label: Nicotine Working Standard
Concentration -
Date -
Technician Name -

3.4.3 Transfer an aliquot from each vial to an auto sampler vial and seal. Set the auto sampler to "maximum" wash between samples. Between each sample or standard vial, place a vial of absolute methanol to act as a solvent wash to prevent sample transfer which occurs at these very low levels.

3.4.4 Standardize the instrument as outlined in this methodology, Section 8. Adjust the attenuator as necessary.

3.4.5 Analyze samples as outlined in Section 9.8.